

CLAIMS

1. Isolated DNA sequence encoding a polypeptide having the biological activity of amorpha-4,11-diene synthase.

2. DNA sequence as claimed in claim 1 which 5 exhibits at least 70% homology to the sequence as shown in Fig. 12 or the complementary strand thereof and which codes for a polypeptide having the biological activity of the enzyme amorphadiene synthase.

3. DNA sequence as claimed in claim 2, which is 10 at least 80%, preferably at least 90%, more preferably at least 95% homologous to the sequence in Fig. 12.

4. DNA sequence as claimed in claims 1-3, which has the sequence as shown in Fig. 12.

5. DNA sequence as claimed in claims 1-4, 15 characterized in that it has been isolated from plants producing amorpha-4,11-diene, for example A.annua and V.oblongifolia

6. Use of a DNA sequence as claimed in claims 1-5 for transforming or transfecting a host cell.

20 7. DNA construct comprising the DNA sequence as claimed in claims 1-5 operably linked to suitable transcription initiation and termination sequences.

8. Host cell comprising a DNA sequence as claimed in claims 1 to 5 or a DNA construct as claimed in 25 claim 7.

9. Host cell as claimed in claim 8, wherein the cell is a bacterial cell, in particular an E.coli cell.

10. Host cell as claimed in claim 8, wherein the cell is a plant cell.

30 11. Host cell as claimed in claim 10, wherein the cell is derived from a plant itself producing sesquiterpenes.

12. Host cell as claimed in claim 11, wherein the cell is an A.annua cell or a V.oblongifolia cell.

35 13. Host cell as claimed in claim 11, wherein the cell is derived from a plant selected from the group

consisting of the genera Carum, Cichorium, Daucus, Juniperus, Chamomilla, Lactuca, Pogostemon and Vetiveria.

14. Host cell as claimed in claim 10, wherein the cell is derived from a plant in which the 5 biosynthesis of sesquiterpenoids can be induced by elicitation.

15. Host cell as claimed in claim 14, wherein the cell is derived from a plant selected from the group consisting of the genera Capsicum, Gossypium, 10 Lycopersicon, Nicotiana, Phleum, Solanum and Ulmus.

16. Host cell as claimed in claim 10, wherein the cell is derived from a plant selected from the group of soybean, sunflower and rapeseed.

17. Host cell as claimed in claim 8, wherein 15 the cell is a yeast cell.

18. Host cell as claimed in claim 17, wherein the yeast cell is a Saccharomyces cerevisiae or Pichia pastoris cell.

19. Host cell as claimed in claim 17, wherein 20 the cell is a oleaginous yeast cell.

20. Host cell as claimed in claim 19, wherein the oleaginous yeast cell is a Yarrowia lipolytica cell.

21. Host cell as claimed in claims 8 and 10-16, which cell is part of a tissue or organism.

25 22. Transgenic tissue, consisting at least part of host cells as claimed in claims 8 and 10-16.

23. Transgenic organism, consisting at least part of host cells as claimed in claims 8 and 10-16.

24. Polypeptide having the biological activity 30 of the enzyme amorphadiene synthase in isolated form obtainable by isolating the polypeptide from A.annua or V.oblongifolia by a process as described in Example 1.

25. Recombinant polypeptide having the biological activity of the enzyme amorphadiene synthase 35 obtainable by expressing a DNA sequence as claimed in claims 1-5 in a suitable host cell as claimed in claims 8-20.

26. Method of preparing amorphadiene,
comprising:

- a) incubating a polypeptide as claimed in claim 24 or 25 in the presence of farnesyl pyrophosphate (FPP) 5 in an incubation medium at a suitable temperature and during a suitable period of time; and
- b) optionally isolating the amorphadiene thus formed.

27. Method of preparing amorphadiene,
10 comprising the steps of:

- a) transfecting or transforming a suitable host cell with a DNA sequence as claimed in claims 1-5 or a construct according to claim 7 to obtain transgenic host cells;
- 15 b) expressing the said DNA sequence in the presence of farnesyl pyrophosphate (FPP) to form amorphadiene; and
- c) optionally isolating the amorphadiene thus formed,

20 wherein the expression level of the amorphadiene synthase is higher in transgenic host cells, tissues or organisms harboring an endogenous version of the DNA sequence than in non-transgenic host cells, tissues or organisms.

28. Method of preparing artemisinin,
25 comprising:

- a) incubation of a polypeptide as claimed in claim 24 or 25 in the presence of farnesyl pyrophosphate (FPP) and the enzymes that further convert amorpha-4,11-diene to artemisinin in an incubation medium at a 30 suitable temperature and during a suitable period of time; and
- b) optional isolation of the artemisinin thus formed.

29. Method of preparing artemisinin,
35 comprising:

- a) transfecting or transforming a suitable host cell, tissue or organism with a DNA sequence as claimed

in claims 1-5 or a construct according to claim 7 to obtain transgenic host cells, tissues or organisms;

b) expressing the said DNA sequence in the presence of farnesyl pyrophosphate (FPP); and

5 c) optionally isolating the amorpha-4,11-diene thus formed,

wherein the transgenic host cells, tissues or organisms harbor the genetic information coding for the enzymes that further convert amorpha-4,11-diene to artemisinin

10 and wherein the expression level of the amorpha-4,11-diene synthase is higher in transgenic host cells, tissues or organisms harboring an endogenous version of the DNA sequence than in non-transgenic host cells, tissues or organisms.

15 30. Source of artemisinin, comprising host cells, tissues or organisms harboring a DNA sequence as claimed in claims 1-5 and the genetic information coding for the enzymes that further convert amorpha-4,11-diene to artemisinin, which host cells, tissues or organisms 20 have expressed the said DNA sequence.

31. Source as claimed in claim 30, wherein the cells are bacterial cells, yeast cells or plant cells.

32. Source as claimed in claim 30, wherein the cells are disrupted.

25 33. Transgenic cell, tissue or organism harboring in its genome more copies of a DNA sequence as claimed in claims 1-5 than are present in a corresponding non-transgenic cell, tissue or organism.

34. Transgenic cell as claimed in claim 33, 30 which cell is an E.coli cell.

35. Transgenic cell as claimed in claim 33, which cell is a Saccharomyces cerevisiae cell.

36. Transgenic cell as claimed in claim 33, which cell is a oleaginous cell, in particular a Yarrowia lipolytica cell.

37. Transgenic organism as claimed in claim 33, which organism is a plant itself producing sesquiterpenes.

38. Transgenic organism as claimed in claim 37,
which organism is A.annua or V.oblongifolia.

39. Transgenic organism as claimed in claim 37,
which organism is a plant selected from the group
5 consisting of the genera Carum, Cichorium, Daucus,
Juniperus, Chamomilla, Lactuca, Pogostemon and Vetiveria.

40. Transgenic organism as claimed in claim 33,
which organism is a plant in which the biosynthesis of
sesquiterpenoids can be induced by elicitation.

10 41. Transgenic organism as claimed in claim 40,
which organism is a plant selected from the group
consisting of the genera Capsicum, Gossypium,
Lycopersicon, Nicotiana, Phleum, Solanum and Ulmus.

42. Transgenic organism as claimed in claim 33,
15 which organism is a plant selected from the group
consisting of soybean, sunflower and rapeseed.